C1 contd

group consisting of SF_6, CF_4, $CBrF_3$, C_4F_8, $CClF_3$, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$, and C_4F_{10}, said microvesicles having resistance against collapse resulting, at least in part, from pressure increases effective when a suspension of said gas-filled microvesicles is injected into the bloodstream of a patient.

2. (Twice amended) A method of making a contrast agent having resistance against collapse from pressure increases when used in ultrasonic echography, said contrast agent consisting of gas-filled microvesicles suspended in an aqueous liquid carrier phase, the microvesicles being microbubbles filled with a physiologically acceptable gas wherein the gas is bounded by a stabilizing layer of one or more film forming phospholipids in lamellar or laminar form at the gas/liquid interface [bounded by an evanescent gas/liquid interfacial closed surface], said method comprising the steps of:

preforming the microvesicles or precursors thereof under an atmosphere of a first gas; and

substantially substituting at least a fraction of said first gas with a second gas which is [a physiologically acceptable said] the physiologically acceptable gas [being] selected from the group consisting of SF_6, CF_4, $CBrF_3$, C_4F_8, $CClF_3$, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$, and C_4F_{10}, said microvesicles having resistance against collapse resulting, at least in part, from pressure increases effective when a suspension of said gas-filled microvesicles is injected into the bloodstream of a patient.

C2

6. (Amended) The method of claim [5] 1, in which at least part of the phospholipids are in the form of liposomes.

C 2 contd

7. (Amended) The method of claim [5] 1, in which at least one of the phospholipids is a diacylphosphatidyl compound wherein the acyl group is a C_{16} fatty acid residue or a higher homologue thereof.

C3

13. (Twice amended) A method of making a contrast agent for ultrasonic echography which consists of gas-filled microbubbles suspended in an aqueous liquid carrier phase, the microbubbles having resistance against collapse resulting from pressure increases effective when the said suspensions are injected into the bloodstream of a patient, and the microbubbles being filled with a physiologically acceptable gas wherein the gas is bounded by a stabilizing layer of one or more film forming phospholipids in lamellar or laminar form at the gas/liquid interface, said method comprising the step of forming the microbubbles in the presence of [a physiologically acceptable gas,] said physiologically acceptable gas [being] selected from the group SF_6, CF_4, $CBrF_3$, C_4F_8, $CClF_3$, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$, and C_4F_{10}, said gas being such that, under standard conditions, the pressure difference ΔP between pressures at which the bubble counts are about 75% and 25% of the original bubble count is at least 25Torr.

C4

15. (Amended) A method of making a contrast agent having resistance against collapse from pressure increases when used in ultrasonic echography, said contrast agent consisting of gas-filled microvesicles suspended in an aqueous liquid carrier phase, the microvesicles being microbubbles filled with a gas mixture wherein the gas mixture is bounded by a stabilizing layer of one or more film forming phospholipids in lamellar or laminar form at the gas/liquid interface [bounded by an evanescent gas/liquid interfacial closed surface], said method comprising the step of forming the microvesicles in the presence of [a] the gas mixture comprising a physiologically acceptable gas[, said physiologically acceptable gas being] selected from the group consisting of SF_6, CF_4, $CBrF_3$, C_4F_8, $CClF_3$, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$ and

C_4F_{10}, said microvesicles having resistance against collapse resulting, at least in part, from pressure increases effective when a suspension of said gas-filled microvesicles is injected into the bloodstream of a patient.

16. (Amended) A method of making a contrast agent having resistance against collapse from pressure increases when used in ultrasonic echography, said contrast agent consisting of gas-filled microvesicles suspended in an aqueous liquid carrier phase, the microvesicles being microballoons filled with a physiologically acceptable gas wherein the gas is bounded by [a] an organic polymer [material] envelope at the gas/liquid interface, said polymer envelope formed from one or more polymers selected from the group consisting of polylactic or polyglycolic acid and their copolymers, denatured albumin, reticulated hemoglobin, and esters of polyglutamic and polyaspartic acids, said method comprising the step of forming the microvesicles in the presence of [a physiologically acceptable gas,] said physiologically acceptable gas [being] selected from the group consisting of SF_6, CF_4, $CBrF_3$, C_4F_8, $CClF_3$, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$ and C_4F_{10}, said microvesicles having resistance against collapse resulting, at least in part, from pressure increases effective when a suspension of said gas-filled microvesicles is injected into the bloodstream of a patient.

17. (Amended) A method of making a contrast agent having resistance against collapse from pressure increases when used in ultrasonic echography, said contrast agent consisting of gas-filled microvesicles suspended in an aqueous liquid carrier phase, the microvesicles being microballoons filled with a gas mixture wherein the gas mixture is bounded by [a] an organic polymer [material] envelope at the gas/liquid interface, said polymer envelope formed from one or more polymers selected from the group consisting of polylactic or polyglycolic acid and their copolymers, denatured albumin, reticulated hemoglobin, and esters of

Sub D1 Cont

C4 Cont

polyglutamic and polyaspartic acids, said method comprising the step of forming the microvesicles in the presence of [a] the gas mixture comprising a physiologically acceptable gas, [said physiologically acceptable gas being] selected from the group consisting of SF_6, CF_4, $CBrF_3$, C_4F_8, $CClF_3$, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$ and C_4F_{10}, said microvesicles having resistance against collapse resulting, at least in part, from pressure increases effective when a suspension of said gas-filled microvesicles is injected into the bloodstream of a patient.

18. (Amended) A method of making a contrast agent having resistance against collapse from pressure increases when used in ultrasonic echography, said contrast agent consisting of gas-filled microvesicles suspended in an aqueous liquid carrier phase, the microvesicles being microbubbles filled with a gas mixture wherein the gas mixture is bounded by a stabilizing layer of one or more film forming phospholipids in lamellar or laminar form at the gas/liquid interface [bounded by an evanescent gas/liquid interfacial closed surface], said method comprising the steps of:

preforming the microvesicles or precursors thereof under an atmosphere of a first gas; and

substantially substituting at least a fraction of said first gas with a second gas which is [a] the gas mixture comprising a physiologically acceptable gas[, said physiologically acceptable gas being] selected from the group consisting of SF_6, CF_4, $CBrF_3$, C_4F_8, $CClF_3$, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$ and C_4F_{10}, said microvesicles having resistance against collapse resulting, at least in part, from pressure increases effective when a suspension of said gas-filled microvesicles is injected into the bloodstream of a patient.

Sub D2

19. (Amended) A method of making a contrast agent having resistance against collapse from pressure increases when used in ultrasonic echography, said contrast agent

Sub D2 Cont

consisting of gas-filled microvesicles suspended in an aqueous liquid carrier phase, the microvesicles being microballoons filled with a physiologically acceptable gas wherein the gas is bounded by [a] an organic polymer [material] envelope at the gas/liquid interface, said polymer envelope formed from one or more polymers selected from the group consisting of polylactic or polyglycolic acid and their copolymers, denatured albumin, reticulated hemoglobin, and esters of polyglutamic and polyaspartic acids, said method comprising the steps of:

preforming the microvesicles or precursors thereof under an atmosphere of a first gas; and

substantially substituting at least a fraction of said first gas with a second gas which is [a physiologically acceptable gas, said] the physiologically acceptable gas [being] selected from the group consisting of SF_6, CF_4, $CBrF_3$, C_4F_8, $CClF_3$, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$ and C_4F_{10}, said microvesicles having resistance against collapse resulting, at least in part, from pressure increases effective when a suspension of said gas-filled microvesicles is injected into the bloodstream of a patient.

20. (Amended) A method of making a contrast agent having resistance against collapse from pressure increases when used in ultrasonic echography, said contrast agent consisting of gas-filled microvesicles suspended in an aqueous liquid carrier phase, the microvesicles being microballoons filled with a gas mixture wherein the gas mixture is bounded by [a] an organic polymer [material] envelope at the gas/liquid interface, said polymer envelope formed from one or more polymers selected from the group consisting of polylactic or polyglycolic acid and their copolymers, denatured albumin, reticulated hemoglobin, and esters of polyglutamic and polyaspartic acids, said method comprising the steps of

preforming the microvesicles or precursors thereof under an atmosphere of a first gas; and

substantially substituting at least a fraction of said first gas with a second gas which is [a] the gas mixture comprising a physiologically acceptable gas[, said physiologically acceptable gas being] selected from the group consisting of SF_6, CF_4, $CBrF_3$, C_4F_8, $CClF_3$, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$ and C_4F_{10}, said microvesicles having resistance against collapse resulting, at least in part, from pressure increases effective when a suspension of said gas-filled microvesicles is injected into the bloodstream of a patient.

25. (Amended) The method of claim [24] 15, in which at least part of the phospholipids are in the form of liposomes.

26. (Amended) The method of claim [24] 15, in which at least one of the phospholipids is a diacylphosphatidyl compound wherein the acyl group is a C_{16} fatty acid residue or a higher homologue thereof.

32. (Amended) A method of making a contrast agent for ultrasonic echography which consists of gas-filled microbubbles suspended in an aqueous liquid carrier phase, the microbubbles having resistance against collapse resulting from pressure increases effective when the said suspensions are injected into the bloodstream of a patient and the microbubbles being filled with a gas mixture wherein the gas mixture is bounded by a stabilizing layer of one or more film forming phospholipids in lamellar or laminar form at the gas/liquid interface, said method comprising the step of forming the microbubbles in the presence of [a] the gas mixture comprising a physiologically acceptable gas[, said physiologically acceptable gas being] selected from the group consisting of SF_6, CF_4, $CBrF_3$, C_4F_8, $CClF_3$, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$ and C_4F_{10}, said gas or at least a gas in said gas mixture being such that, under standard conditions,

the pressure difference ΔP between pressures at which the bubble counts are about 75% and 25% of the original bubble count is at least 25Torr.

33. (Amended) A method of making a contrast agent for ultrasonic echography which consists of gas-filled microballoons suspended in an aqueous liquid carrier phase, the microballoons having resistance against collapse resulting from pressure increases effective when the said suspensions are injected into the bloodstream of a patient and the microballoons being filled with a physiologically acceptable gas wherein the gas is bounded by an organic polymer envelope at the gas/liquid interface, said polymer envelope formed from one or more polymers selected from the group consisting of polylactic or polyglycolic acid and their copolymers, denatured albumin, reticulated hemoglobin, and esters of polyglutamic and polyaspartic acids, said method comprising the step of forming the microballoons in the presence of the [a physiologically acceptable gas, said] physiologically acceptable gas [being] selected from the group consisting of SF_6, CF_4, $CBrF_3$, C_4F_8, $CClF_3$, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$ and C_4F_{10}, said gas or at least a gas in said gas mixture being such that, under standard conditions, the pressure difference ΔP between pressures at which the bubble counts are about 75% and 25% of the original bubble count is at least 25Torr.

34. (Amended) A method of making a contrast agent for ultrasonic echography which consists of gas-filled microballoons suspended in an aqueous liquid carrier phase, the microballoons having resistance against collapse resulting from pressure increases effective when the said suspensions are injected into the bloodstream of a patient and the microballoons being filled with a gas mixture wherein the gas mixture is bounded by an organic polymer envelope at the gas/liquid interface, said polymer envelope formed from one or more polymers selected from the group consisting of polylactic or polyglycolic acid and their copolymers,